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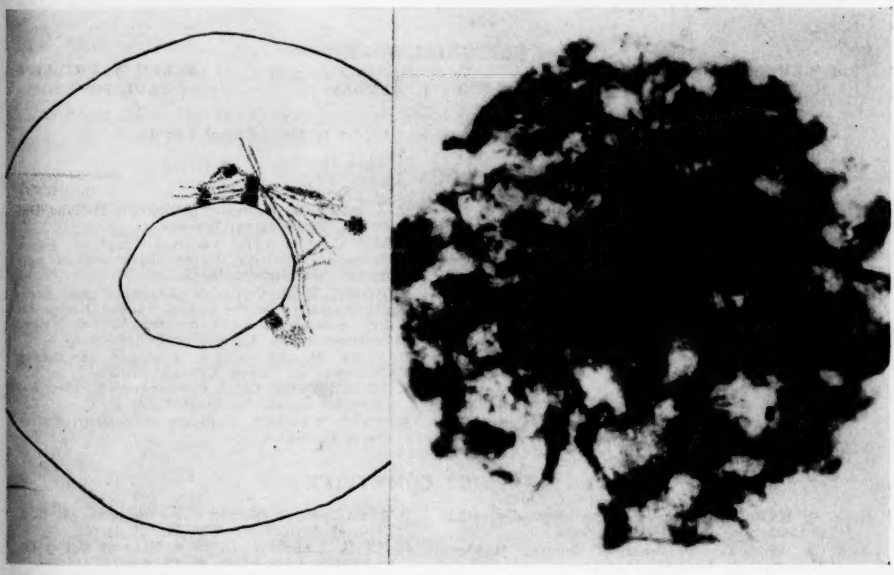

MANY STRANDED CHROMOSOMES IN ENDOSPERM TISSUE

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An Inherited Tumor in *Drosophila*

A Hairless Mutation in the Asiatic Mouse

A New Instance of Multiple Sex-Chromosomes

Gastro-intestinal Tumor in Three Sets of Half-Siblings

"Shaker"—A Sex-Linked Nervous Disorder in the Fowl

VOLUME 41 OCTOBER, 1950 NUMBER 10

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An incorporated organization devoted to promoting a knowledge of the laws of heredity and their application to the improvement of plants, animals, and human racial stocks

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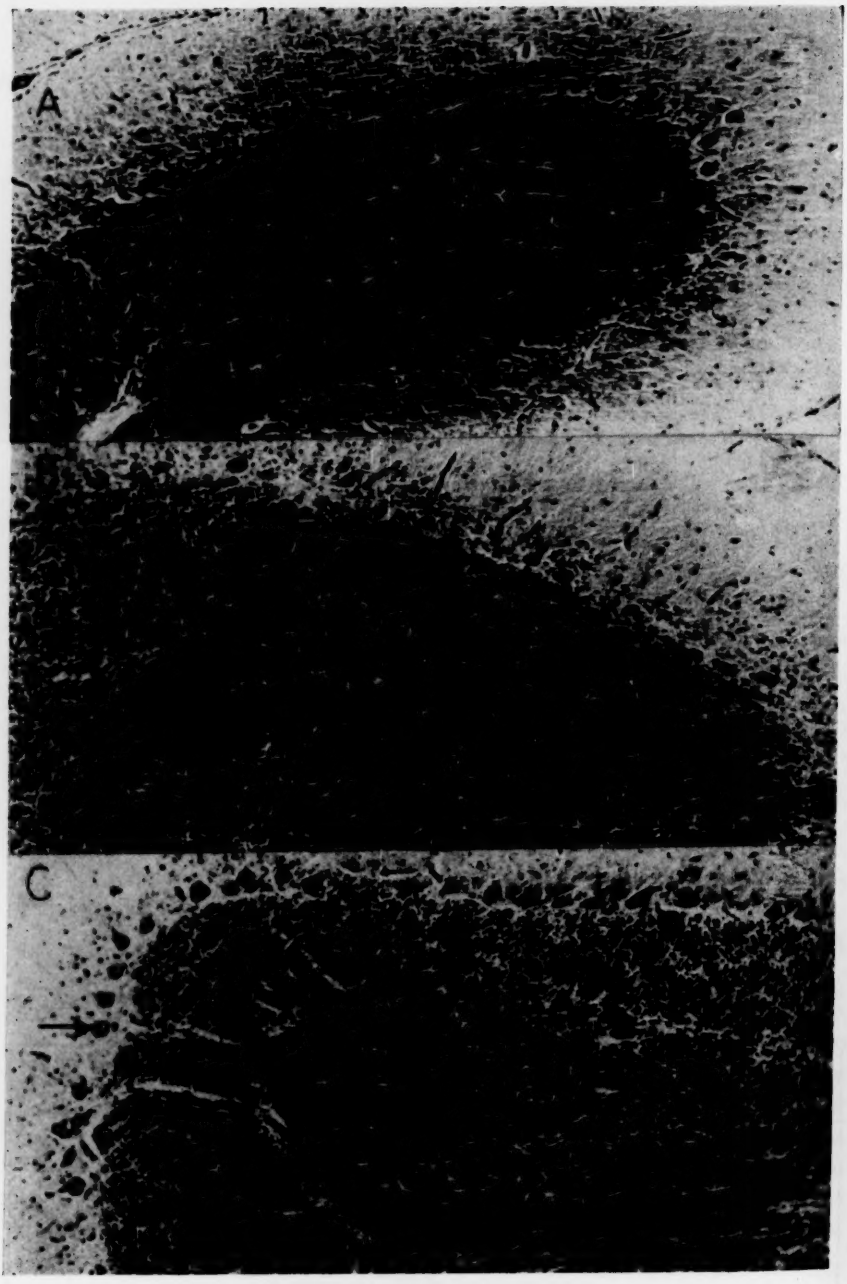

CEREBELLAR DEGENERATION

Figure 1

Sections from cerebella of chickens. *A*—with well developed "Shaker" symptoms, *B*—with incipient "Shaker" symptoms, and *C*—with no symptoms (normal control). The "Shaker" symptoms are accompanied by a degeneration of the Purkinje cells (one is shown by an arrow in *C*). Note almost complete loss of Purkinje cells in *A*, and the partial disappearance, and shrinkage and increased intensity of staining of those which remain in *B*. Stained with Hematoxylin and eosin. 100×

THE "SHAKER" FOWL

A Sex-Linked Semi-Lethal Nervous Disorder

H. M. Scott, C. C. Morrill, J. O. Alberts and Elmer Roberts

Illinois Agricultural Experiment Station

In the routine examination of stock hatched for replacement purposes in the spring of 1948, a nervous disorder was noted which was not wholly unlike the clinical symptoms of infectious avian encephalomyelitis (epidemic tremor) in which there is a rapid vibration, of small amplification, in the head and cervical regions. The symptoms also could readily be confused with those of Newcastle disease in the early stages of the nervous phase of this disease. However, laboratory tests were negative for Newcastle virus. An examination of the pedigree hatching records disclosed that all afflicted individuals originated from a single mating involving sire G2596 and sixteen distantly related mates, all of the Rhode Island Red breed and all apparently normal in action. This particular mating was one of thirty-two single sire matings made in 1948 in which White Leghorns, Barred Columbians and Barred Plymouth Rocks were involved as well as Rhode Island Reds.

Each of the sixteen females involved in the original mating with sire G2596 produced defective progeny. From this mating there were 332 individuals that survived to eight weeks of age at which time they were classified as to sex and the "Shaker" condition. At the close of the regular breeding season, G2596 was mated to a group of White Leghorn females, eleven of which produced the progeny listed in Table I, mating #2. Fifteen x-bred males from this second mating, all normal in appearance, were in turn tested in single sire matings. Of the fifteen males tested in this manner, five were shown to carry the "Shaker" gene and the distribution of their progeny is recorded in mating #3.

A complete summary of the three matings is given in Table I. None of the 226 male progeny from the three matings exhibited the "Shaker" condition whereas 128 of the 241 female progeny were classified phenotypically as "Shakers." The data suggest that a sex-linked recessive gene is responsible for the "Shaker" phenotype and that the original male, G2596, was heterozygous for this factor.

The nervous disorder is not discernible in the newly hatched chick. A mild but rapid movement of the head and neck has been observed as early as the eighteenth day, but most of the chicks can be classified accurately by the end of the fourth week. The population from mating #2 was carefully observed for the time of onset of the symptoms. Ten of the eleven afflicted individuals had definitely become "Shakers" by the twenty-eighth day. The one remaining chick did not start to shake until after the thirty-fifth day (Table II). With advancing age the shaking becomes increasingly severe as indicated by the severity score. Relatively few "Shaker" individuals were able to walk without stumbling after reaching the age of ten weeks, so violent had become the shaking movements. Excitement tended to aggravate the condition. A majority of the afflicted chicks were unable to stand beyond the age of fourteen weeks and death resulted from inanition. However, a few females were reared to sexual maturity.

Knowlton[2] has described a nervous disorder in newly hatched chicks that is inherited as an autosomal recessive.

Afflicted chicks seldom survived beyond the first week. It was designated by Knowlton as congenital loco, since the chicks were unable to stand upright without toppling over backward. Hutt and Child[1] have reported on the existence of a semi-lethal factor in newly hatched chicks which is characterized by tremors. The vibrations per unit of time decreased with age and finally disappeared entirely. A majority of the chicks did not survive beyond the first week. The mutation was designated as congenital tremor and appeared to behave as an autosomal recessive. Riddle[4] has reported a condition in pigeons which he tentatively designated as hereditary ataxia. Affected birds showed many degrees of lack of muscular control, nodding and swaying of head and neck, unsteady gait and somersaulting backward or forward. The symptoms were generally exaggerated under excitement or fear. He stated that the condition appeared, with some irregularities, as a Mendelian recessive. It is clear that the "Shaker" condition described herein, adds a third member to the group of nervous disorders in the fowl having a hereditary basis. It is also apparently distinct from the condition in pigeons observed by Riddle, at least in respect to mode of inheritance.

Histopathological Findings

Histopathological examination* of tissues, particularly of the central nervous system, was made on 20 birds. Of these, 14 were shakers and six were non-shakers or controls. Of the controls, four were from the same matings which produced shakers (two males and two females) and two were from unrelated normal stock. Tissues were fixed in Zenker's fluid and in 10 percent formalin. Zenker-fixed tissues were embedded in tissuemat and stained with hematoxylin and eosin. Formalin-fixed tissues were prepared according to Mahon's technique[3] for the demonstration of myelin.

One lesion which was consistently present in the affected birds and absent in the controls was a significant loss of cerebellar Purkinje cells with degeneration of many of those remaining (Figure 1*A* and *B*). The cells to be destroyed last appeared to be those of the gyri nearest the base of the cerebellum. Degeneration was manifested particularly by shrinkage and increased intensity of staining of the cells. Limited evidence of neuronal degeneration was seen in the ponsmedulla of a few birds. In no case was evidence of significant demyelination demonstrated.

An occasional and inconsistent finding was limited focal and perivascular infiltration of cerebrum and cerebellum with leucocytes, predominantly lymphocytes. These were seen generally in the white matter or stem rather than in the cortex. During this study, an outbreak of Newcastle disease had appeared in the flock and vaccination with a live virus vaccine had been carried out. It is believed that the mild inflammatory lesions encountered in some of the "Shakers" was due to exposure to Newcastle disease virus (either the field strain or the vaccine), for two reasons: (1) some of the control birds showed these lesions, and (2) some of the "Shakers" examined earlier did not. If the infiltrations are not a "Shaker" lesion, that fact would help differentiate the "Shaker" from a bird affected with infectious encephalomyelitis, since birds with encephalomyelitis generally develop such infiltrations. Failure to demonstrate in "Shakers" the presence of the hyaline capillary thrombi and areas of necrosis characteristic of encephalomalacia assists in the differentiation of these two diseases.

The primary lesion in "Shakers" therefore appears to be a degeneration and ultimate necrosis and disappearance of cerebellar Purkinje cells unaccompanied by significant demyelination or inflammatory changes. This is in harmony with the accepted view that the

TABLE I.—Classification of progeny resulting from test matings

Matings #	Male Progeny Normal	Male Progeny Shaker	Female Progeny Normal	Female Progeny Shaker	Total
1	165	0	78	89	332
2	27	0	12	11	50
3	34	0	23	28	85
Totals	226	0	113	128	467

TABLE II.—The onset and severity of shaker symptoms in the eleven afflicted females from the second mating

	Age in days 21	28	35	42
No. of afflicted chicks	3	10	10	11
Av. severity score*	1.0	1.19	1.70	2.73

*1.0 = slight, 2.0 = moderate, 3.0 = severe

*We are indebted to Miss Emily L. Dorstewitz for the preparation of tissues for histopathological examination.

Purkinje cells have a function related to muscular coordination. The significance of the lesion is further strengthened by the fact that the severity of symptoms varied directly with the extent of Purkinje-cell destruction (Figures 1*A* and *B*). Unfortunately, in none of the other inherited nervous disorders cited herein was the brain examined histopathologically, so comparisons cannot be made.

Literature Cited

1. HUTT, F. B. and G. P. CHILD. *Jour. Hered.* 25:341-350. 1934.
2. KNOWLTON, F. L. *Oregon Agr. Expt. Sta. Bull.* 253. 1929.
3. MAHON, G. F. *Arch. Neurol. and Psychiat.* 38:103. 1936.
4. RIDDLE, OSCAR. *Proc. Soc. Expt. Biol. and Med.* 15: 56-58. 1917.

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A HAIRLESS MUTATION IN ASIATIC TAME MICE

IN 1937 a hairless male appeared at this university in a colony of common tame mice with black spotted coat. Since 1934 the mice have been bred without any special care in our aviary. Their origin is not certain, but it seems probable that they are the domesticated variety of the Asiatic mouse, *Mus molossimus*.

Experiments with this mutant mouse yielded the following data: mating of the hairless male with normal females produced five litters consisting of 34 individuals, 19 males and 15 females, all normal haired.

Matings of F_1 females with the hairless male yielded 27 offspring, 15 haired and 12 hairless. Matings of an F_1 male with normal females produced 11 offspring, all haired.

These data show that hairless in this case is a simple recessive like similar mutations in the house mouse and in the Norway rat. As in those species, the hair begins shedding when the animal is between two and three weeks old. Hairless young are very delicate and difficult to rear. Hairless females in general show a very low fertility and consequently the hairless stock could not be maintained.

Sections of hairless skin show that it differs from that of normal mice in having a much thickened cutis, though the epidermis is normal in appearance.

A hair follicle of the hairless skin has epithelial cells normal in appearance, but in the follicle there is no papilla. From such a follicle the hair has been shed and its papilla

ASIATIC HAIRLESS MICE

Figure 2

Two juvenile hairless mice and a young adult showing the hairless mutation which first appeared in 1937. As in the house mouse of Europe and America, hairless is recessive.

SURFACE AND SECTION OF HAIRLESS SKIN

Figure 3

The surface view (left) shows the presence of hair pits as black spots. But no hairs are present. The section of the skin (right) shows the non-functional follicles. 100×.

has undergone degeneration.

These histological observations are in agreement with those of Crew and Mirskaia on the hairless house mouse, *Mus musculus*, reported in *Journal of Genetics* 25:17-24, 1932.

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◆

A New Pedigree of Otosclerosis

Pfandler (*Schweizerische medizinische Wochenschrift* 79:692-704, 1949) states that there is an irregular dominance, with perhaps simple recessiveness, for otosclerosis. Geneologic investigations and detailed clinical studies on an extremely prolific farmer family in Switzerland, which had lived in the same region for centuries, and in which there had been consanguineous marriages, indicated that otosclerosis is a distinct and characteristic nosologic entity, which is brought on by a genetic mutation with dominant character. It is believed that in the heterozygous state otosclerosis rarely becomes manifest; if it does, it is less severe and appears only at an advanced age. This is explained by the extremely variable expressiveness and the feeble penetrating power of the involved hereditary factor. Even when the factor does not manifest itself clinically for several generations, this does not justify the assumption of a recessive heredity because the histologic examination of the organ of hearing reveals the existence of otosclerotic foci in about 10 per cent of the population.—*J.A.M.A.* 142:67, 1950.

THE NUCLEUS IN DIFFERENTIATION AND DEVELOPMENT

III. Nuclei of Maize Endosperm

ROBERT E. DUNCAN AND JAMES G. ROSS*

NUCLEI from the central area of maize endosperm in the milk stage of development are of a larger size than those of the peripheral area, and by 25 days after pollination may have developed a diameter four or five, and occasionally up to ten, times greater. The calculated volumes of the central nuclei are therefore roughly 64 or 125 and in a few extreme cases 1000 times that of the volume of the peripheral nuclei. This presents a problem similar to that which has interested animal cytologists for many years. Jacobj[9] and others measured the variability in nuclear size in many mammalian tissues and concluded that they fall into size classes. Others have speculated that the size classes arose through successive doubling of the nuclear material with intervening growth periods which accounted for the size classes not being a regular geometric progression. Geitler[4] in particular has assessed nuclear size in relation to endomitosis as have Huskins and Steinitz[7] and Huskins and Bennett[8] who have also reviewed the literature on endomitosis and polysomaty in both plants and animals.

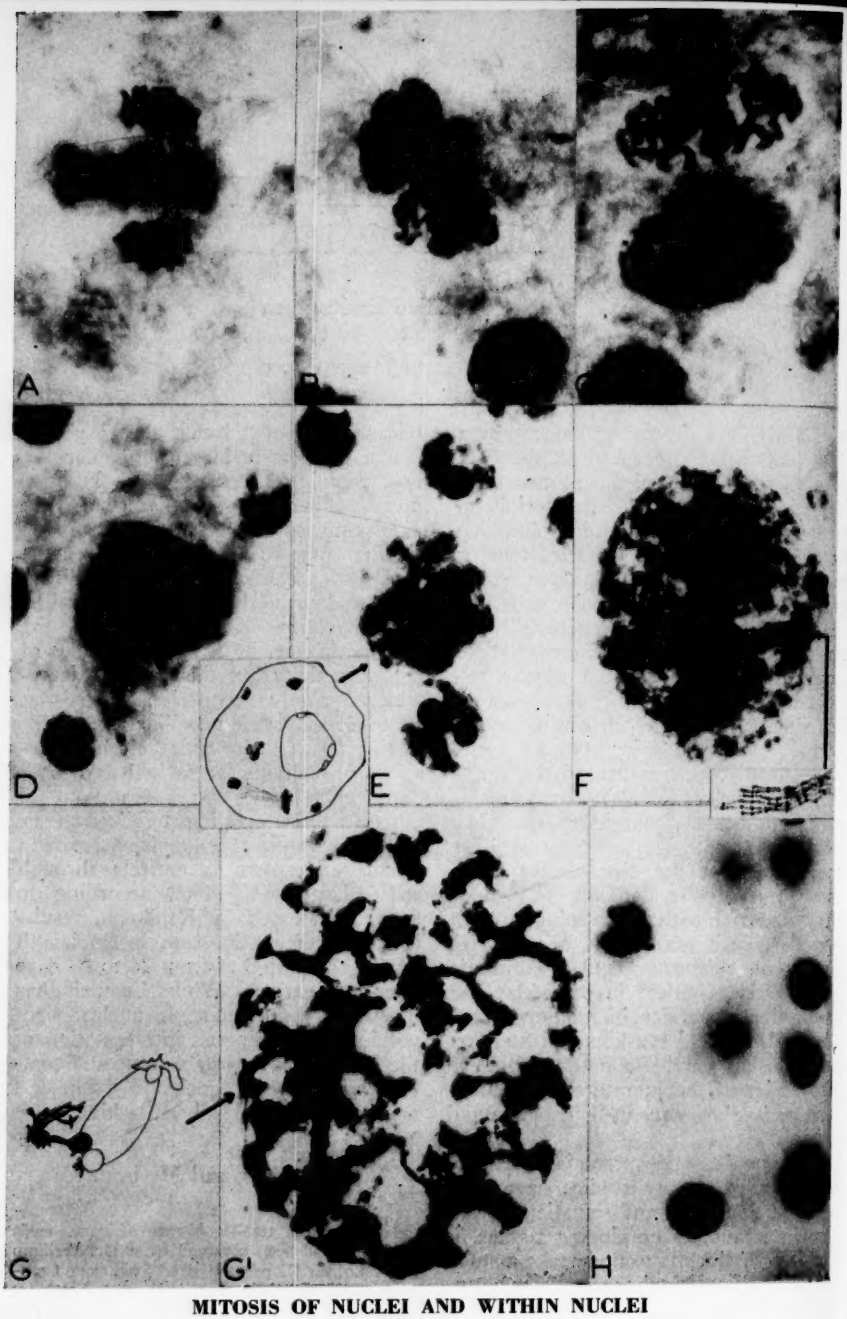
Since maize endosperm is a triploid tissue and so many studies have been made of mutations and somatic segregation in it and its enveloping tissues, it seemed worthwhile to undertake a study of the cytological changes which occur in the endosperm.

Randolph[12] stated that the caryopsis of maize grows in length and diameter most rapidly between 10 and 25 days after pollination. He described the endosperm as being free-nucleate for the first four to seven nuclear generations and becoming completely cellular by the fourth day. The thin-walled parenchymatous cells composing the endosperm are, at first, all capable of mitotic activity but this capacity is retained by only the peripheral cells at 16 to 18 days after pollination. Cells of the abgerminal surface of the endosperm may divide for several days later than elsewhere. After cessation of mitotic activity in these localized areas, except for sporadic cell divisions, growth of the endosperm is entirely through cell enlargement, which according to figures presented by Randolph, results in a 50 percent increase in its length during the period of from 24 to 48 days after pollination. While Lampe[10] has illustrated the diversity in nuclear sizes within the endosperm, interpretation of the increase in nuclear volume and comparative study of nuclei of peripheral and interior cells has been lacking.

Materials and Methods

Kernels of a two-knob race of corn, seeds of which had been obtained from Dr. William Brown of the Pioneer Hi-Bred Seed Corn Company were fixed in 3 : 1 absolute-acetic at 3, 6, 10, 14, 21, and 25 days after close (with-

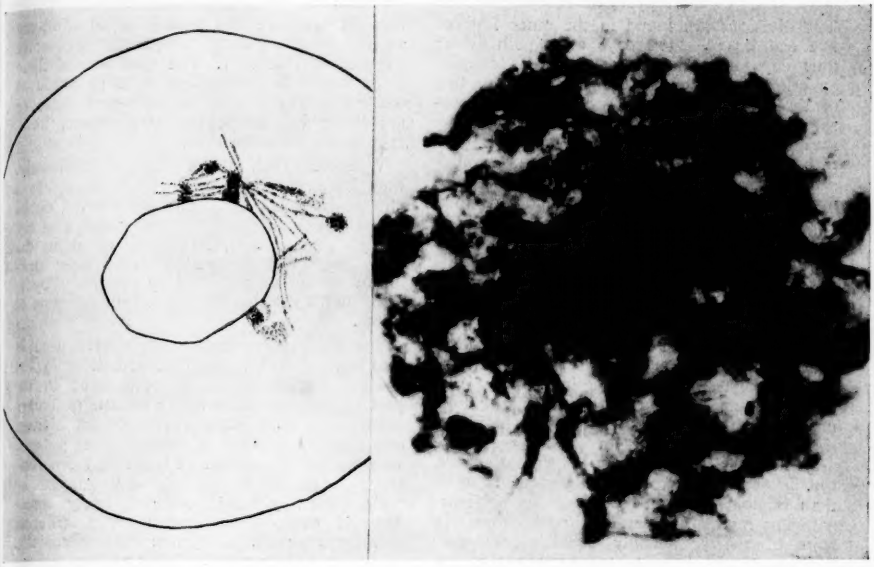
*Research Associate and Research Assistant (now Assistant Professor of Agronomy, Brookings, South Dakota) respectively, Department of Botany, University of Wisconsin. The Program in Cytology is supported in part by grants from the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation. The authors wish to thank Y. H. Tung, A. Owczarzak, and M. D. Persidsky for their aid in preparing the illustrations; N. G. Johnson and O. M. Huskins for making many of the measurements and calculations; and M. K. Schraufnagel for making some of the preparations.



MITOSIS OF NUCLEI AND WITHIN NUCLEI

Figure 4

Some aspects of mitosis and endomitosis in maize endosperm. *A-G* are from acetocarmine squashes; *H*, from sections stained by the Feulgen technique. *A-E* and *H* are from endosperm 10 days after pollination; *F* from 14-day-old, and *G* from 20-day-old endosperm. 1125×. The individual photographs show the following features: *A*—early telophase nuclei from peripheral zone; and *B* a later telophase from this region. *C* telophase nuclei from a zone intermediate between regions where mitoses do and do not occur. *D* is an endosperm wherein 30 chromosomes are present. *E* shows nucleus with quite dispersed chromatin. Here six homogeneous areas represent condition of knobs in energic nuclei. The knob indicated by an arrow has four strands departing from one side. *F* is a nucleus with relatively dispersed chromatin. The rope-like strand to the right is made up of four to six finer strands which are thicker than those in *E*. An endoprophase-like nucleus is shown at *G'*. The chromosome of type 6 drawn in detail at the left (*G*) shows at least 10 constituent strands. *H* shows nuclei from the peripheral zone; prophase at lower right, prometaphase at upper left.



MULTI-STRAND CHROMOSOMES

Figure 5

A flattened nucleus from acetocarmine squash of endosperm, 14 days after pollination. One of the chromosomes of type 6 is shown in detail in the diagram of the nucleus at the left. 1125×.

in the race) pollination. They were either squashed in aceto-carmine or embedded, sectioned and stained by the Feulgen technique.

Measurements of the diameter of nuclei were made in sectioned material. The localized areas selected for study, where nuclei were measured, were 1) the adgerminal layer of endosperm cells lying between the embryo and the adjacent enveloping tissues, 2) the outermost layer of endosperm cells on the abgerminal side, and 3) the cells in the central area of the endosperm. Generally, nuclei of adjacent cells (ten per section in each of ten successive sections) from the region of the endosperm being investigated, were selected for measurement. If a nucleus, however, had visible chromosomes or was one which had an irregular shape so that its volume would be difficult to calculate, it was excluded and an adjacent suitable nucleus of the tissue was chosen. The nature of the geometric figure which best expressed the shape of any particular nucleus was determined by measuring its width, length, and depth. The loci presented in Figure 6*C* represent the average of the measurements of diameter of 100 nuclei from each area sampled. Since the depth measurements of nuclei which were elliptical in outline were equal to the minor diameters, such nuclei have the characteristics of prolate spheroids. The volumes and surfaces of spherical and spheroidal nuclei were calculated according to standard formulas. The results of these calculations based on the average for each sample are presented in Figure 6*A* and *B*, volumes and surfaces respectively.

Observations

There was not sufficient material of the early fixations (three and six days after pollination) to allow determination and study of the onset of the variability in size occurring among the endosperm nuclei. At 10 and 14 days, however, new cells are still being added in sufficient numbers in the outer zones of the endosperm so that a comparative study was possible of nuclei obviously capable of division and of the large energic nuclei which have not been observed to divide again. No mitoses were seen in the 25 day material; cells of the outer layer are more radially elongated than in younger material.

The cells from the outer layer of endosperm, 10 or 14 days in age, have the ability to divide in both tangential and radial planes. In addition, cells lying deeper within the endosperm divide in these and planes oblique to them,

The divisions are found in the outer four or five cell layers at 10 days after pollination; four days later, however, the area occupied by cells capable of division extends only two or three cells below the surface of the endosperm. These nuclear divisions seem to be normal (Figure 4*A* and *B*). The plates are rather crowded so that the number of chromosomes cannot be determined easily in sectioned material; but 30, the normal triploid complement for maize endosperm, are found in squashes.

The newly formed sister nuclei resemble each other at first (Figure 4*B*) but the similarity can be lost since they may differ increasingly as they grow in size. The outer one arising from a mitosis whose spindle is radially oriented may appear to be in the energic condition while the inner may have the appearance of a nucleus in prophase (Figure 4*C*). The number of nucleoli in either sister nucleus is usually three at first (Figure 4*B*) but later only one may be present. Here, too, is evidence that neither nucleus is more than triploid since there is one nucleolar-organizing region per set of chromosomes. In spite of the presumably equal chromosome endowment the second type of daughter nucleus may reach a volume roughly twice that of its sister although, in some cases, they may still lie in the same cell.

Nuclei which contained well defined chromosomes that appear conspicuously larger than those in adjoining cells are found in some cells within the inner part of and just within the region where mitosis occurs. These nuclei are more or less spherical and have diameters approximately equal to the major axis of the elipsoid nucleus described as containing distinct chromosomes (Figure 4*C*). Such nuclei (Figure 4*D*) are generally solitary; their respective sisters are so dissimilar in size and structure that they cannot be recognized easily.

These large spherical nuclei may have more than twice the diameter of the prophase nuclei of the outer cells. Their chromosomes may be as much as four times as broad as the chromosomes in those cells, and are arranged loosely at the inner surface of the nuclear membrane. The nucleolar components commonly are resolved into a single large nucleolus or into a larger and a smaller one; in any case they stain quite darkly with carmine. Such nuclei are broken easily in making squash preparations and the scattered chromosomes can be counted without error. Thirty have been present in all those examined. Thus an approximate doubling in nuclear diameter without a doubling in chromosome number, but accompanied by a change in chromosome girth, has ocurred. This is the last convincing chromosome count that can be made in an enlarging nucleus. These chromosomes seem not unlike prophase chromosomes except that they have greater girth than at prophase and metaphase in the zone where mitoses occur frequently (compare Figures 4*C* and 4*H*). The condition of these large nuclei is reminiscent of a prophase of the endomitotic cycle; no endo-metaphase or anaphasic and telophasic congressions, however, have been observed.

Whatever such nuclei may do ultimately, they have not been observed to complete a mitotic division cycle. The neighboring nuclei in the intermediate zone of the endosperm are energic and smaller, larger, or of about the same size. Only the smaller ones appear capable of mitosis as indicated by relative proportions of the division figures within the zone of tissue.

The knobbed strains of corn were used in the hope that the knob contribution to the energic nucleus could be recognized in the part of the endosperm where mitosis no longer occurs and their number provide an indirect check on the number of chromosomes present. In some energic nuclei (Figure 4*E*) six relatively large bodies can be distinguished by their relatively intact outlines, fairly homogeneous composition, and slightly different staining reaction. They are heterochromatic, as shown by Morgan[11]. They seldom are applied against the surface of the nucleolus as the nucleolar organizer of a chromosome #6 generally is. In certain phases of nuclear activity there are six irregularly outlined, darkly stained regions, which can be interpreted as knobs. Six groups of similarly-staining granules are present in a few large nuclei and suggest by their association that each group of granules represents an aspect of a knob. The relationship between the two aspects, however, is not evident. In still other nuclei there may be as many as 30 bodies which corresponds to the normal number of chromosomes (Figure 4*G*).

The presence of six knobs indicates that the expected triploid number of chromosomes is present. They also serve as markers in following out and counting the strands constituting the chromosomes involved. In one of the clearer cases studied six relatively homogeneous knobs were present in the nucleus. One favorably located knob (Figure 4*E*) had at least four strands emerging from one side. Presumably these strands represent replications of the chromonemata. This particular nucleus has a diameter roughly twice that of a nucleus from the peripheral region of the endosperm.

The presence of six clearly defined knobs often can not be determined easily in larger nuclei since there may be many smaller darkly-staining granules which obscure the structures interpreted as knobs in smaller nuclei. A nucleus at this time generally is a more or less hollow prolate spheroid (Figure 4*F*) with a single large nucleolus or two nucleoli, a larger and a smaller one, lying next to the nuclear

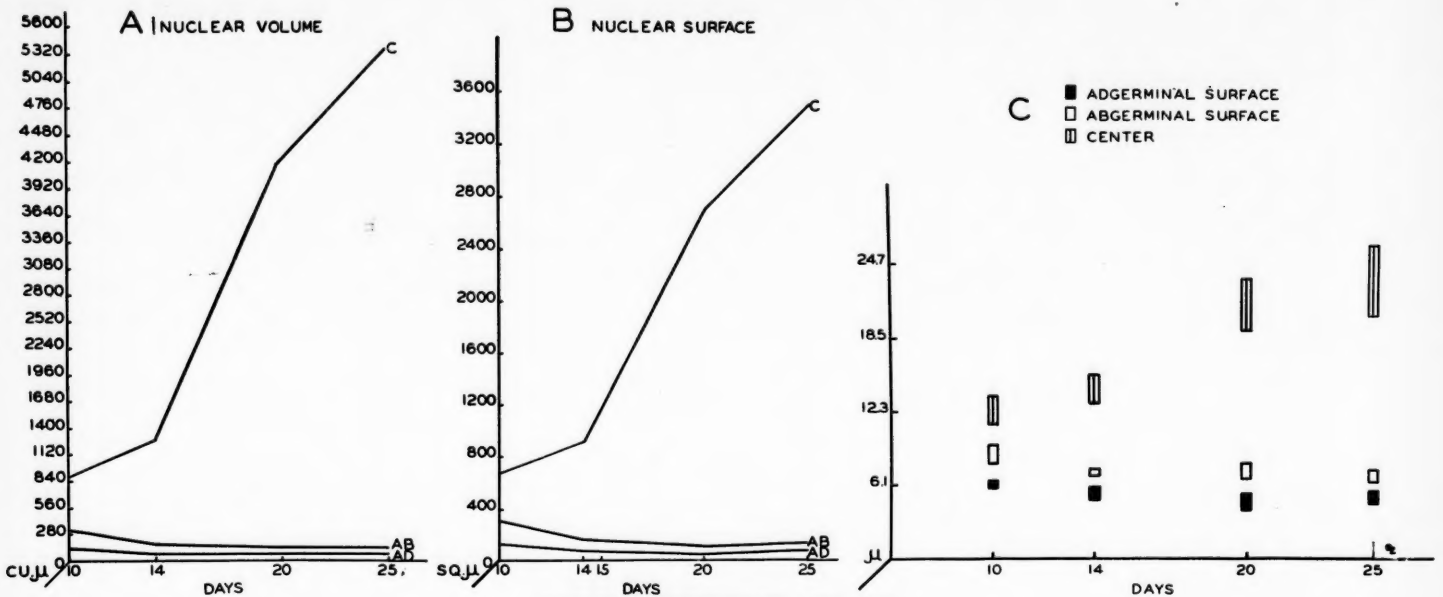
membrane and projecting into the central plenum. The central area of the nucleus is, to all appearances, free of carmine, orcein, or Feulgen-positive material after staining by the usual procedures. The outer zone of a large nucleus, however, is exceptionally rich in such material. The chromatin appears as rope-like strands, each made up of finer strands, after aceto-carmine. The rope-like strands vary in thickness, partly, at least, in relation to the number of constituent strands. Four to six relatively coarse strands make up different portions of the group lying on the surface facing toward the right of the nucleus in Figure 4*F*. On the basis that at least four strands are distinguishable the number of strands making up this chromosome is not greater (though they are coarser) than the number attached to one side of a knob in the nucleus illustrated in Figure 4*E* (four strands). Other strands which can be followed in the same nucleus are single or four-parted possibly because some chromonemata lie adjacent to their sisters for their entire lengths and others diverge from this orientation.

Some nuclei from samples of older endosperm (Figure 4*G* from 20 days after pollination) are characterized by comparatively coarse strands which can be homologized with chromosomes on the basis that about 30 are present and only three are attached to the nucleolus. The strands vary in width along their length, and may terminate in an area of fine granules or in a series of larger granules. In addition, granules may be inserted within a finer strand, which may form a portion of the chromosome or lie alongside it. Some chromosomes, such as a #6, identified by its attachment to the nucleolus in Figure 4*G*, and drawn in detail at the left of that photograph, can be followed throughout a portion of their length so that some detail of their structure can be observed. The sketch of this particular chromosome shows a lack of association, resembling an unravelled thread, between components of a portion of the chromosome where at least 10, possibly 16, strands are present. The chromosome as a whole is longer than a #6 from a nucleus in the prophase of mitosis in the regions and age of endosperm where mitosis occurs. Its component strands are of variable and different girths; the narrowest are approximately as wide as the whole chromosome in mitotic figures. The variability in girth along the length of this chromosome, as distinct from that brought about by variable association of component strands, is illustrated by the portion of the chromosome near the attachment to the nucleolus. Here the component strands are associated and are not distinct. The appearance of other chromosomes in this nucleus suggests they are composed of many strands. The volume of this particular nucleus is roughly 100 times larger than a nucleus from the peripheral region of endosperm of the same age. The appearance of chromatic material suggests that this nucleus may be a manifestation, in an older nucleus, of an endomitotic condition similar to that in Figure 4*D*. It is not believed that the number of strands which can be counted necessarily represents a true census of the number of chromonemata making up a chromosome but only that it is indicative of the multiplication occurring. Figure 5 illustrates the complexity of a chromosome of type #6 in a large nucleus from 14 day endosperm.

Several attempts were made to induce divisions in maize endosperm at the milk stage by use of the treatments employed by Huskins and Steinitz[7], but no mitoses were seen in the treated material. There seemed, however, to be a certain rearrangement of chromatic material as though some preparation for the appearance of prophasic chromosomes were in progress. One is led to believe that nuclei with conspicuously 8—16 stranded chromosomes do not undergo mitosis even under such unusual stimulation.

More than three nucleoli have not been observed in any of these large nuclei; frequently two of dissimilar size are present. When there is a single nucleolus, the surface of the nucleolus has been searched for the heterochromatin segments associated with the nucleolus-organizers of a chromosome of type #6. The number varies; one, two, or three may be present depending upon the extent to which fusion has occurred. The diameter of a nucleolus resulting from the activity of two or three nucleolar organizers (Figure 4*F* and *G*) may exceed that of an entire triploid nucleus (Figure 4*H*) in the peripheral region where mitosis occurs and is many times that of a comparable nucleolus from that region. One of the more common aspects is a sphere with two knobs of unequal size, one obviously double (Figure 5). These facts are indications that such nuclei are not more than triploid, in terms of chromosomes and disregarding the number of strands therein.

The relative dimensions of nuclei in selected parts of the endosperm are presented in Figure 6*C*. The extremes of each bar represent respectively the averages of the longer and shorter diameters of the ellipsoid optical sections of 100 nuclei. As far as the averages of nuclear diameter presented in Figure 6 are concerned, the nuclei in the selected areas on the adgerminal and abgerminal surfaces of the endosperm have similar changes in nuclear diameter; they are smaller on the twentieth than on the tenth day and a bit larger on the twenty-fifth than the twentieth day afer pollination. The nuclei from the adgerminal surface cells, however, are smaller than those from the outer layer of cells on the abgerminal side. The diameters of nuclei



NUCLEAR DIMENSIONS AND AGE

Figure 6

Changes with age, of nuclear proportions in three regions of the endosperm. *A*—nuclear volume, calculated from averages in Figure 6*C*. Line *C* represents nuclei from the central region; line *AB*, nuclei from the adgerminal surface; and line *AD* nuclei from the adgerminal surface. Graph *B* shows the change in nuclear surface between 10 and 25 days calculated from averages in 6*C*. The letters represent the same entities as in the preceding figure. *C* shows changes in nuclear diameters with age. The extremes of the bar represent the longer and shorter diameters (average of 100 energic nuclei from as continuous a series of cells as possible) respectively. The squares represent samples of spherical nuclei.

from the central area of the endosperm roughly are doubled from the tenth to the twenty-fifth days. As the nuclei from the central region increase in size their eccentricity increases; this is illustrated by the increase in length of the bars in Figure 6*C*.

In addition to the nuclear change described, the cytoplasm in many cells undergoes alterations which have to do with the development of its storage function. The region of the cytoplasm immediately outside the membrane of the centrally located nucleus of a cell not yet storing starch may appear granular. This zone in which the granules are most frequent is stained mildly following treatment by the Feulgen technique (Figure 4*H*). It is in this region that starch grains appear at about the fourteenth day after pollination. Later they may still lie in this region but they may be so closely packed in older endosperm cells that no vestige of the original distribution remains. The early distribution of starch grains was first brought to our attention by Dr. M. J. Wolf of the Northern Regional Laboratory of the U.S.D.A.

Discussion

Growth in size of a nucleus such as occurs in maize endosperm, has often been associated with an increase in chromatin content of the individual nucleus. Evidence for an increased amount of chromatin can be found either in a doubling (and, perhaps, subsequent redoubling) of the chromosome number or in an increase in the number of strands making up each chromosome. The degree of strandedness can, of course, be determined accurately only where the number of strands making up a chromosome can be counted. In other cases where there is considerable growth of the nucleus after the last satisfactory count of the number of strands per chromosome, only a general estimate of the degree of strandedness and its relation to nuclear size is possible. Buck[1], for example, has associated increase in proportions of salivary gland chromosomes of *Sciara* with increase in nuclear volume and Cooper[3] has stated that the largest chromosomes are found in the largest salivary gland cells of the *Diptera*. It is generally accepted that such chromosomes are highly multistranded.

Increase in nuclear size in maize endosperm parallels (1) the increase in dimensions of the strands or (2) the number of discernible strands making up a chromosome or (3) both. In addition the contrast in size of the nucleoli in the large nuclei of older endosperm and in the smaller nuclei from regions of the endosperm where mitoses occur presents evidence, somewhat similar to that for the chromatin, of an increase in the amount of nucleolar materials. Doubling of the volume (Figure 6*B*) requires five to six days during the period of time from 10 to 25 days after pollination. It is not known whether a doubled volume necessarily represents a size class associated with the intervals at which each of a series of levels in strandedness is completed. The composition of the sample of centrally located nuclei from the various ages of endosperm becomes increasingly diverse; as seen in classifications in Figure 7. The magnitude of the growth suggests that there is a great increase in nuclear components, perhaps greater than accounted for by the observed increase in discernible strands per chromosome.

The increase in the average diameter, surface, and volume of nuclei (Figure 6 *C*, *B*, and *A* respectively) from the central region of the endosperm, when plotted against time, follows a sigmoid curve whose grand phases of development occur in close agreement with that for elongation of the endosperm and caryopsis.[12] Since all the nuclei of endosperm with the exception of those in the outer few rows of cells grow in size markedly, the nuclei from the sample of the central region are more or less representative of the nuclei of the cells which constitute the major part of the endosperm. Agreement between the time of initiation and, to some extent, the duration of growth phases of the endosperm tissue with those of the average nucleus from the central region is possible, provided a fairly close correlation of nuclear and cell volumes is maintained throughout this change.

During the same period of time that nuclei from the central part of the endosperm and the endosperm tissue itself

are in their grand phase of growth, the process of storing starch is initiated and developed. When plastids containing young starch grains are first detected, they are distributed throughout the cytoplasm but are most conspicuous just outside the nuclear surface. Thus its area, which is a function of nuclear volume and shape, seems of importance in this phase of cell activity. Storing starch, however, is only one of the activities of the endosperm. It need not be expected, therefore, that the greatest amount of starch be found in the cells with the largest nuclei. Further evidence of the importance of the area of the nuclear surface is found in the fact that the nuclei are spheroidal rather than spherical and in that the nucleoproteins are localized at the nuclear surface as observed in both fixed and fresh materials. When the volumes of the nuclei are larger, as they are in older endosperm, the eccentricity of the average nucleus is greater. The effect of this increase in eccentricity on the area of nuclear surface contributes, to some degree, to restraining the rate of decrease in the ratio of nuclear volume to nuclear surface.

The relation of increasing nuclear volume to tissue growth and nuclear surface to cell activities suggests a possible role of increase in strandedness. Geitler[4] has suggested that endomitosis provides a method for continued cell growth and activity without interference from the process of mitosis. The importance of the increase in number of strands, however, may be more far-reaching. Huskins[6] has pointed out a possible relationship between the gene replication entailed in endomitosis and gene activity above and beyond self reproduction.

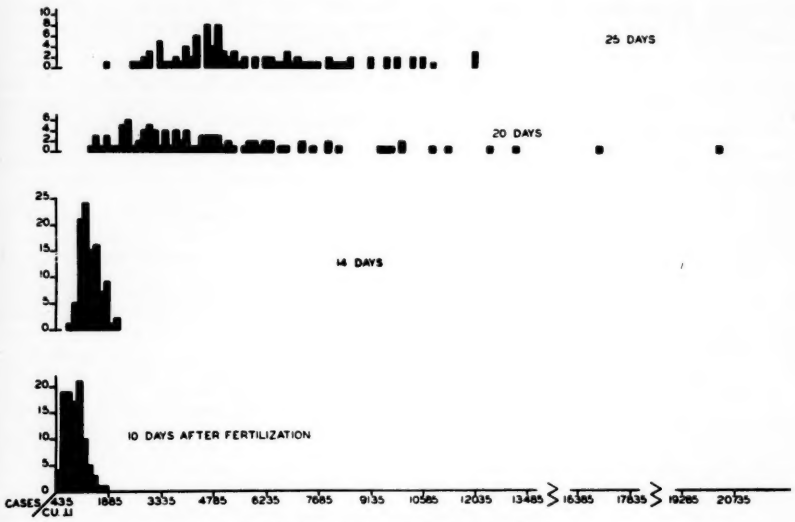
The relation of the increase in number of strands to the localized areas of color or storage material within the endosperm is somewhat problematic. It is evident, however, from the illustration in Clark and Copeland's paper[2] that regions of the endosperm where nuclei are greatly enlarged may be involved in loss of an endosperm character (*Wx* in this case) and that chromosome aberrations are found in regions where mitoses occur.

Nuclei of the outermost cell layers of the endosperm behave quite differently from the nuclei of the central region in that the former grow but little during the first twenty-five days after pollination, and retain mitotic activity longer. This difference in growth leads to considerable differences in size between nuclei from the center and from the periphery. Even within the peripheral layer there are localized areas where the nuclei are smaller than elsewhere in the surface layer. These same areas are those where mitotic activity persists longest.

A comparison between volumes of younger and older endosperm nuclei of a peripheral region which can be followed with some certainly is possible. Such a region is the one between the embryo and the adjacent enveloping tissues. There apparently is a reduction in size from the tenth to the fourteenth day after pollination. This observation must be treated with reserve but it may indicate that investigation of the relationship between nuclear size and (1) age of tissue and (2) mitotic activity would be profitable.

Another size difference between nuclei in the peripheral layer exists between the nuclei of the abgerminal surface and those of the layer of endosperm between the embryo and the enveloping tissue. The nuclei from the latter surface are smaller; the disparity is present both when mitoses are occurring and after they cease. Perhaps it can be attributed to some phase of embryo-endosperm relationship such as competition for the raw materials of nuclear components.

While there are small changes in dimensions of the nuclei of surface layers, when comparisons of the older and younger homologues are made, further investigation of these differences is necessary in order to assess their statistical significance.



VOLUME CHANGES WITH AGE

Figure 7

Variations in volume of nuclei from the central region of endosperm. The size classes are arbitrary. The increasing nuclear volume with advancing age of the tissue is very evident.

It is interesting that there is a larger proportion of spherical nuclei in the regions where mitoses take place. Since mitoses occur in the cells from which the 10 day adgerminal and 10 and 14 day abgerminal samples were taken, the small changes in nuclear diameters in this tissue may, in part, be a reflection of a relationship between mitoses and nuclear shape.

Recently Geitler's description[5] of the endosperm of *Gagea* has come to hand. He described the large endosperm nuclei as having arisen through endomitosis which accounts for their polyploid condition. Their structure is characterized by large homogeneous heterochromatin masses, which are greater in size than the chromocenters of energic nuclei in the embryo. They obscure the large irregularly shaped nucleoli and the inconspicuous scattered euchromatin. At prophase the heterochromatin seems to flow into the chromosomes as they appear. The large nuclei are highly polyploid. On the other hand when the nuclei of maize endosperm increase in size there is no increase in chromosome numbers. The enlarged nuclei are associated with increase in multi-strandedness of chromosomes. Geitler reported that the nuclei in *Gagea* endosperm, may diminish in size and appear denser, presumably as a result of dehydration of the endosperm as it matures. Depolyploidization, however, was suggested as a possible alternative. This diminution is comparable to that which occurs in older maize endosperm but is not comparable to diminutions in size of nuclei in tissues where mitoses occur.

Endomitosis in maize endosperm is a derived and secondary phase of growth exhibited by cells in a period of specialized physiological activity. On the basis that the inner nucleus of a pair of sister nuclei at the peripheral region of the endosperm may be the only one to enlarge without undergoing mitosis later, a differentiation, if no more than of location, occurs at anaphase. It seems

likely that a dissimilarity between sister telophase nuclei (Figure 4*C*) is further expression of this difference in location.

After endomitosis has become established as the method of nuclear growth, the endosperm nuclei are quite variable in the extent of dispersion of chromatic material. Furthermore, a nucleolus may assume one of a considerable number of aspects. When the chromatin is relatively dispersed it may appear as spherical. When the chromosomes are more distinct, a nucleolus may be elongated, a little smaller, and be less retentive of stain. When about 30 chromosomes can be counted, it may be impossible to see the nucleolus, either because it is not present or it is hidden by the chromosomes. Perhaps such alternations in the state of chromatin and nucleolar materials are phases of the endomitotic process. As the nuclei grow, the diameters and lengths of the chromosomes become progressively greater than those of prophase chromosomes from regions where mitoses occur.

Summary

Changes in nuclear size and activity were followed in developing *Zea Mays* endosperm at 3, 6, 10, 14, 20, and 25 days after either sib or self-pollination of a line of maize which had two heterochromatic knobs per chromosome set. The major and minor diameters of 100 energic nuclei in 100 adjoining cells (10 per section in each of 10 successive sections) in each area of the 10, 14, 20, and 25 day endosperms were measured and the respective measurements averaged. These figures were used in calculating the average volume and surface of the nuclei in each area. Nuclei in the adgerminal layer of surface cells were generally smaller than those on the abgerminal surface. In both these areas a reduction in nuclear size from the 10 to 14 day interval after pollination was indicated. In neither of these areas did the nuclei appear to increase in size subsequently. The nuclei in the central area of the endosperm, on the other hand, grew up to 1000 times their original volume in the 24 days subsequent to pollination. The grand phase of this growth occurred in close agreement with that of the endosperm as a whole and with the inception and development of starch storage. This nuclear growth was an endomitotic process in which the number of strands per chromosome and the breadth and length of the individual strands increased. The number of knobs and of nucleolus organizers per nucleus remained constant; this is evidence that the number of chromosomes per nuclens does likewise. The amount of nucleolar material increased greatly. The increase in amount of nuclear surface and in number of chromonemata per chromosome is discussed in relation to the physiological specialization of the endosperm cells.

Literature Cited

1. Buck, J. B. *Proc. Nat. Acad.* 23:423-428. 1937.
2. Clark, F. J. and E. C. Copeland. *Am. Jour. Bot.* 27:247-251. 1940.
3. Cooper, K. W. *Proc. Nat. Acad.* 24:452-458. 1938.
4. Geitler, L. *Oester. Bot. Zeitschr.* 95:277-299. 1948.
5. ————. *Chromosoma* 3:271-281. 1948.
6. Huskins, C. L. *Amer. Nat.* 81:401-434. 1947.
7. ———— and L. M. Steinitz. *Jour. Hered.* 39:67-77. 1948.
8. ————and R. L. Bennett, in press.
9. Jacobj, W. *Arch. Entwicklungsmech.* 106:124-192. 1925.
10. Lampe, Lois. *Bot. Gaz.* 91:337-376. 1931.
11. Morgan, D. T., Jr. *Jour. Hered.* 34:194-198. 1943.
12. Randolph, L. F. *Jour. Agric. Res.* 53:881-916. 1936.

THE INHERITANCE OF A TUMOR

In *Drosophila melanogaster**

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DESPITE the fact that hereditary tumors in *Drosophila melanogaster* were first noted by Bridges in 1916, our knowledge concerning the genetics of these phenomena remains very scant today. Several investigators have endeavored to analyze the inheritance of tumors in various strains[2,6,7,8,9,11,12], but only two, Russell and Ghelelovitch, have achieved any measure of success.

Russell, by a technique of substituting marked chromosomes carrying crossover inhibitors, was able to demonstrate that the tumor factor in the *st sr e^s ro ca, tu-36a,* strain is located on the second chromosome and that modifying factors are present on the first and third chromosomes. Ghelelovitch's recent description has indicated that the fat body tumor depends on a genetic factor located on the left arm of chromosome two and that modifiers are present on the third, and possibly the fourth chromosomes.

While these works represent significant advances, they do not give us the loci of the tumor factors, nor do they indicate whether both tumors depend on the same factor, or on different factors.

There are actually two major difficulties standing in the way of genetic research on *Drosophila* tumors. First is the general low tumor incidence to be found in the various *tu* strains. This may be due to our inability, through ignorance of their nature, to produce consistently high incidence strains by selection, inbreeding, or other means. Second, there is a marked tendency for incidence to fluctuate spontaneously even in closely inbred strains.

Thus one is confronted with the problem of having no stable, consistently high-incidence material on which to run an analysis. Also the development of high incidence strains awaits, to a great extent, the very knowledge which can be gained only through genetic investigation.

The present paper details certain findings regarding the genetic background of one tumor strain. These findings were made possible by a chance change of constancy (and later, of rate) of tumor incidence in the *bw tu* stock under culture in our laboratory.

The tumors in this strain are not restricted to any particular organ or region, but appear most commonly in the abdomen and thorax. In the general sense they are bengin and follow the same course as the majority of other *Drosophila* tumors. They appear during early larval development and by late second instar show signs of pigmentation. Pigmentation develops through the third instar and by pupation, the entire tumor mass is completely encapsulated. Cell degeneration follows encapsulation and by the time of adult emergence an apparently inert mass of black pigment is all that remains. Occasional misshapen adults will emerge, showing large pigment areas at the bases of rudimentary, deformed, or missing appendages. It has not been determined in these cases whether the deformity is the result of an invasive growth into the imaginal discs or merely the effect of a mechanical pressure or blocking by the pigment mass.

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Observations and Experimental Data

The *bw tu* stock was obtained from cultures maintained at the California Institute of Technology. Upon receipt, individuals were examined for black masses and a tumor incidence slightly in excess of 35 percent was recorded. The stock was inbred through ten generations to render it reasonably homozygous, and reexamined for tumor incidence. Following inbreeding, incidence was found to be approximately 38 percent. These figures on incidence, as are the ones to follow, are for uncontrolled room temperature varying in the extreme over a range of 10° C.

In the first year following the establishment of the inbred stock, incidence was moderately consistent within a range of 5 to 10 percent. Occasional wider fluctuations were noted, but these were not frequent.

In the late spring of 1948, however, the stock showed a spontaneous and very sudden rise in incidence to approximately 75 percent. This was followed in a few generations by a decline to 40 percent. In the several months which followed, it was found that the stock had become unusually stable in its 40 percent incidence and showed only small fluctuations from this value. So consistently minor were these fluctuations that there appeared some hope that partial, if not complete success might reward attempts at a genetic analysis.

As a first step, a series of reciprocal outcrosses to an isogenic wild strain (*Burlington, Vt.*) were made. The F_1 flies resulting were all phenotypically wild and showed no tumors. F_2 offspring (from flies chosen at random among the F_1 generation) showed the following segregation:

Total flies	852
Wild	614
Wild-*tu*	19
bw	158
bw tu	61

Males and females appeared in approximately equal numbers in all classes.

These data show a typical Mendelian segregation of the *bw* factor, as would be expected. The segregation of tumors may therefore be considered directly.

Throughout this experiment constant checks revealed that the inbred *bw tu* stock was maintaining its approximate 40 percent incidence. Assuming that this 40 percent incidence represents partial penetrance of a homozygous recessive character and that such a rate of partial penetrance would hold through the above experiment, one would expect to find 85 tumorous flies in a total F_2 of 852, since 85 represents 40 percent of the theoretically expected recessives in a 3-1 segregation. Moreover, the fact that the large number of tumors remains associated with brown eyes in the F_2 is strongly suggestive of linkage. There is then, the indication that tumors are dependent on a recessive character having limited penetrance, and that this factor is located along with the gene for brown eyes on the second chromosome.

The procedure next indicated was to outcross with strains bearing one or more chromosome II recessives, and to ascertain what data might be gathered from linkage and crossover studies.

Since the *bw* factor is well out on the right arm of the chromosome, the amount of linkage shown in the foregoing experiment indicates that the tumor factor is probably proximal to *bw* in terms of the centromere but not actually too close to the centromere. This being the case, the factor *vg* at locus 67 appeared a logical first choice for crossover studies.

The tumor strain was crossed with a nontumorous, homozygous, *vg* stock and the offspring were mated to secure an F_2 generation. F_2 progeny were examined, flies of the phenotype vestigial-brown-tumor were selected, and cultures developed from them.

Immediately after the establishment of the new stock it was found that tumor incidence was exceptionally high, running at approximately 90 percent. The consistency in terms of percent incidence shown by the *bw tu* stock was not apparent in the new strain, however, and fluctuations as high as 95 percent and as low as 80 percent incidence were recorded. Although the fluctuation was disappointing, the opportunity offered by the higher value for reasonably accurate mapping was indeed fortunate.

Two series of outcrosses to the wild *Burlington* strain, followed by F_1 backcrosses were set up. The results in terms of crossover values for the three factors appear in Table I.

From these data much information can be drawn, even though calculations, failing 100 percent incidence, cannot be exact.

The position of the tumor factor is evidently in the region between *bw* and *vg*. Published maps indicate this distance as being equal to

TABLE I.—Crossover percentages—wing-tumor-eye

Factors involved	Total flies *showing crossover*	% *crossover*
A) Series I. Total Flies: 572		
bw—*vg*	191	33.3
bw—"*tu*"	148	25.8
vg—"*tu*"	129	22.5
B) Series II. Total Flies: 617		
bw—*vg*	238	38.7
bw—"*tu*"	182	29.4
vg—"*tu*"	160	25.9
C) Combined results. Series I and II. Total Flies: 1189		
bw—*vg*	429	36
bw—"*tu*"	330	27.7
vg—"*tu*"	289	24.3

38 units. The calculation of the distance on the basis of the combined results in Table I indicates 36 units. This would seem close enough to check the basic accuracy of the over-all data. The *bw*-tumor crossover is calculated at 27.7 percent and the *vg*-tumor crossover is calculated at 24.3 percent. Adding these gives a value of 52. This is far in excess of the 38 units actually existing, but it is less far off than results which might be obtained by adding either 27.7 or 24.3 to the calculated 36 units found between *bw* and *vg*. Moreover the disparity of 14 units from expectation on the basis of actual map distance probably represents in part the phenomenon of interference and in part the inaccuracy inherent in the fact that the tumor factor for some reason fails to express itself or be recognized in approximately 10 percent of the cases. Thus it seems logical to assume that the alignment of factors from the centromere is vestigial, tumor, brown.

It would further appear that the region in which the tumor factor lies is somewhere between the loci 76.8 and 91. This is determined by adding 24.3, the percent crossover value between *vg* and tumor, to 67, the locus of *vg*, and subtracting 27.7, the percent crossover between *bw* and tumor from 104.5, the locus of *bw*. By doing this one finds a zone of overlap of 14.2 units from 76.8 to 91. Since calculations of both these values which overlap involve the tumor factor, both are probably an equal amount in error. Hence as a working hypothesis it was assumed that the locus of the tumor factor lay in the exact center of the "overlap zone" or around locus 83.9.

This now had to be checked experimentally. To do this a series of outcrossees was made involving the *vg* tumor *bw* stock, and stocks showing recessive mutations between the loci 72.5 and 99.2, on chromosome II. These were run to the F_2 and the F_2 progeny were examined for crossovers between tumor and the other factors. Obviously, the closer the tumor factor lay to a known recessive, the less would be the possibility of crossing over between them, and conversely, the farther apart the two factors lay, the greater would be the possibility of crossing over. The factors used in these test crosses were chubby, 72.5; curved, 75.5; four-jointed, 82.0; welt, 87; humpy, 93.3; and arc, 99.2.

Of the various factors thus studied in preliminary runs, crossing over was recorded with chubby, curved, humpy, and arc, but not with four-jointed at 82.0 or with welt at 87.0. These factors lie fairly close to the center of the "overlap zone" and are thus highly significant as markers.

A repetition of the outcrosses to the *fj* and *wt* strains was made on a more extensive scale. In all, 2157 F_2 flies from the tumor-*fj* cross were examined, and 2200 F_2 flies from the tumor-*wt* cross. No crossover was found between tumor and *fj*, but one fly showing welt-eye and an abdominal tumor was discovered.

From these observations, it was assumed that the tumor factor must lie slightly nearer to *fj* at 82.0 than to *wt* at 87. The region 82-85.5 is therefore indicated rather than 84.5-87. Returning now to the center of the "overlap zone" as calculated on the basis of the original crossover, study, we find 83.9 indicated. The correspondence between this value and the region 82-84.5, in spite of the necessarily indirect experimental procedure, is thus quite good.

It may therefore be postulated that this tumor is dependent upon a recessive factor located at approximately 83.9 on the right arm of chromosome II.

Discussion

The foregoing data are of interest in several connections.

First, although there is agreement with Russell and Ghelelovitch in indicating the second chromosome as the one carrying a tumor factor, there is not agreement with Ghelelovitch in regard to the position of the factor. The tumor factor studied in these experiments seems to be on the right arm of the chromosome, while that described by Ghelelovitch is on the left. This suggests two different tumor factors, or at least two distinct genes having to do with the appearance of tumors. Such a suggestion is strengthened by the fact that Ghelelovitch describes the tumor which he studied as a new one, restricted in location to the fat bodies. No such restriction is to be noted in the case of the tumor under consideration here.

Russell's data permit of no speculation as to the location of the *st sr* tumor factor. Since, however, the tumor in this strain is not restricted, it probably does not depend on the same factor as that indicated by Ghelelovitch.

The suggestion of more than one tumor factor calls attention to the need for exact terminology in designating the genetic factors involved. Current practice employs the symbol *"tu"* to indicate the presence of hereditary tumors in a stock. This symbol is not specific, however, and therefore does not seem apt for the indication of a known and par-

ticular tumor gene. Hence it would appear correct to drop the symbol *"tu"*, and assign a specific label and symbol to each tumor factor when it has been identified. In accordance with this suggestion the designation melanin tumor-*A* is proposed for the tumor factor here under consideration, with the assigned symbol mt^{A}. Subsequently-identified non-localized melanin tumors can be designated by other letters, mt^{B}, mt^{C}, etc., and those which are localized can be described according to their location and morphology. The tumorous vestigial brown-eyed stock studied here might thus be listed *vg* mt^{A} *bw*, while the stock from which it was derived, now known as *bw tu*, might better be listed mt^{A} *bw*. Such procedure will serve to identify and set apart not only the known tumors one from the other, but also from those of unknown genetic origin.

The question as to why an inbred stock homozygous for the mt^{A} or other tumor recessive does not show 100 percent incidence, remains outside the immediate scope of this paper. Investigations of environmental factors[3,4,5] have demonstrated that they are important in influencing tumor incidence, but we have yet to find how they become operative. Both Russell and Ghelelovitch have indicated that modifying genes also have an important role in the question of tumor development. Certainly the addition of some enhancing factor or the elimination of a suppressor seems implicit in the great change in incidence following the crossing of the brown-eyed tumor strain with vestigial, as noted in the previous section. In this case there was no change in culture method or other environmental factor to which it might be ascribed. The analysis of the modifiers therefore, is another field in which far more investigation is necessary.

Summary

An inbred tumor strain of *Drosophila melanogaster, bw tu*, has been analyzed genetically to ascertain the nature and locus of the tumor factor. The analysis has been accomplished in several steps.

An outcross to a wild strain and study of the F_2 progeny reveals the nature of the factor as a recessive and indicates its position as being on chromosome II. The strain *vg* tumor *bw* was created, and orthodox linkage studies made with this strain indicate an approximate locus of 83.9. The position cannot be exactly calculated since tumor incidence is not 100 percent. Therefore the tumor strain was outcrossed with several different strains having homozygous recessives on chromosome II in the region 72.5-99.2. Examination of F_2 flies from these crosses revealed several crossovers between tumor and factors not too close to 83.9; only one crossover between tumor and welt (87); and none between tumor and *fj*, located at 82. The estimated locus of 83.9 on chromosome II is therefore regarded as being reasonably close.

The specific designation melanin tumor-*A*, mt^{A}, is suggested for the tumor factor which has been isolated.

Literature Cited

1. Bridges, C. B. *Genetics.* 1:1-52. 1916.
2. Ghelelovitch, S. *Comptes Rendus des Seances de L'academie de Sciences.* 230:20; 1002-1004. 1950.
3. Hartung, E. W. *Cancer Res.* 2:12: 837-840. 1942.
4. ————. *J. Exp. Zool.* 106:2: 223-232. 1947.
5. ————. *Science.* 107:2777; 296-297. 1948.
6. Russell, E. W. *Genetics.* 27:612-618. 1942.
7. Stark, M. B. *J. Canc. Res.* 3:3. 1948.
8. ————. *Proc. Nat. Acad. Sci.* 5:573-580. 1949a. *J. Exp. Zool.* 27:509-529. 1949b.
9. ————. *Collected papers N. Y. Med. Coll. and Flower Hosp.* 1:397-400. 1935.
10. ————. *Am. J. Canc.* 31:253-267. 1937.
11. ———— and C. B. Bridges. *Genetics.* 11:249-266. 1926.
12. Wilson, I. T. *Genetics.* 9:343-362. 1924.

CARCINOMA OF THE GASTRO-INTESTINAL TRACT

In a Utah Family*

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MANY papers have been published during the past century concerning families with a high incidence of cancer. The literature contains pedigrees and descriptions of these families. Within the past decade statistical studies have been made of the incidence of malignancy in comparison with controls from the same population. These studies have been designed to determine whether or not an hereditary predisposition exists for certain types of cancer.

The literature contains a record of several specific types of precancerous and cancerous conditions which appear to be inherited. *Polyposis intestini*, a predisposing factor for carcinoma, has been described as an inherited disease dependent upon a dominant gene with varying degrees of penetrance.[2,4,5] Retinoblastoma is another malignant disease which generally follows the pattern of a dominant with variable penetrance.[1,11] Neurofibromatosis is considered to be a dominant trait in most cases with irregular inheritance in a few pedigrees.[6,3] Many workers have made statistical surveys of the incidence of breast cancer in families.[7,8,9,10] Their results show that heredity plays an etiological role in predisposition to breast cancer.

The present paper concerns a kindred of polygamous origin that has a high incidence of carcinoma of the gastrointestinal tract (Kindred No. 133 in the Laboratory of Human Genetics, University of Utah). The kindred was brought to the attention of the writers by a great grandson of the original progenitor. An investigation followed which included interviews with members of the family, examination of death certificates and medical records, and interviews with physicians who attended the people.

History of Kindred 133

The pedigree chart (Figure 8) represents four generations of kindred 133. The fifth generation is not included since the children are all very young. The total number of individuals included in the five generations at the present time is 628. The darkened squares and circles symbolize individuals who have died from cancer or have been diagnosed as having cancer. The squares and circles with crosses indicate individuals who are deceased, and the squares and circles with diagonal lines indicate the individuals who are now under forty-five years of age. The diamonds with enclosed numbers in generation IV represent the total number of children, both male and female in each sibship.

The original progenitor (I-1) was born in Denmark in 1834 and emigrated to Utah in 1852 as a convert to the Church of Jesus Christ of Latter Day Saints. He was the only child in the family and nothing is known of his ancestors. Upon arriving in Utah he settled in a small farming community at the base of the Wasatch mountains in the central part of the state. He remained in this community for many years but later moved to a larger farming community on the opposite side of the mountain range. A large number of his descendents still reside in the first settlement and in nearby communities.

Soon after I-1 arrived in the territory of Utah he married a girl (I-2) who was also

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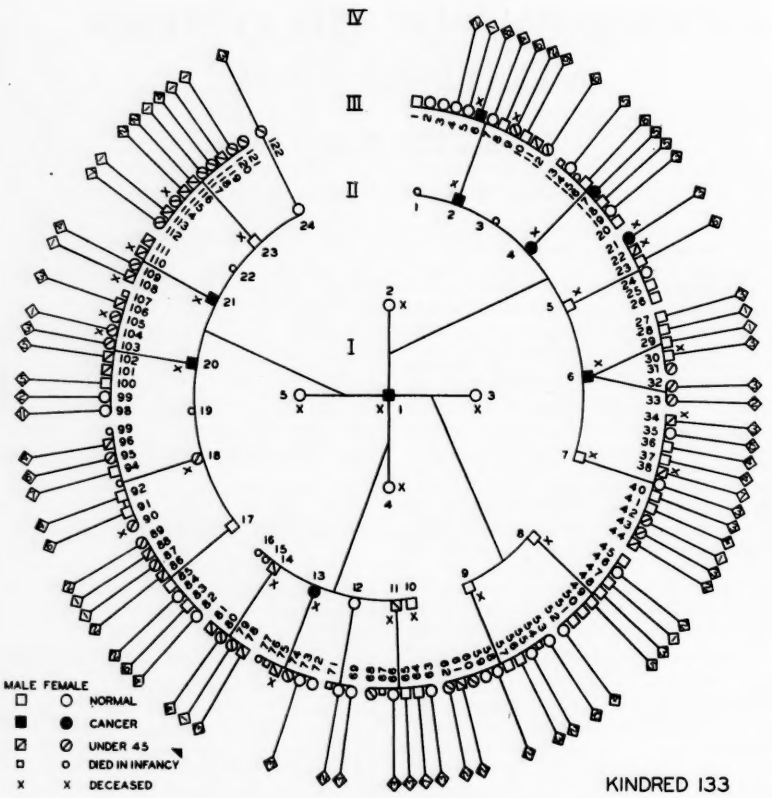

INHERITANCE OF GASTRO-INTESTINAL CANCER
Figure 8

Descendants of four half-sibships originating in polygamous marriages in early Utah show a high incidence of cancer for two generations. With advancing age other cases may yet appear in the third generation.

of Danish ancestry, and within a few years married three other girls of the same ancestry (I-3, 4, and 5). With his family he lived in the two farming communities until the time of his death in 1909. He became the father of twenty-four children. Six of his children died in infancy. A few years before his death he was involved in a horse and buggy accident while driving over a mountainous road. From the accident he received a bruise near his ankle. He always maintained that the bruise was the cause of his cancer, for soon after, a large ulcerated sore appeared in the same area as the bruise. He often showed the sore to his children and grandchildren and in the Danish language would say, "This may be the peg to my casket." The physician who attended him is still alive. He reports that the cause of death was a melanotic growth which originated in the lower leg and metastasised to the inguinal region. He does not believe the intestinal tract was involved.

Two years after the death of I-1, his son (II-2) died from carcinoma of the rectum at the age of 53. His case was diagnosed by the family physician. No operation was performed. The next member to be diagnosed with cancer was II-4. She died from peritonitis in 1930 following an operation for carcinoma of the omentum and sigmoid flexure. She was 59 at the time of death. Seven years previously she underwent an operation for carcinoma of the stomach. Unfortunately the pathological record of this first operation was destroyed by the hospital and the exact nature of the operation is not known. II-6 died from

carcinoma of the stomach in 1934 at the age of 69. The cancer was inoperable when first detected by his physician.

II-5 died in 1908 at the age of 45. This was a year before his father (I-1) died and three years before his brother (II-2) died with cancer. For many years before his death he had serious difficulties with his stomach, but since he lived on an isolated farm many miles from the nearest town, medical attention was not available. Therefore the nature of this disorder is not known. Those who associated with him say his symptoms were similar to those of the other members who died with cancer. It is now a family opinion that he died with a cancer in his stomach. The physician who attended him at the time of death in 1908 is still alive, and although he was not well acquainted with the case, he does not believe II-5 had a carcinoma at the time of death. His death certificate, signed by this same physician, states that he died from respiratory difficulties.

The remaining son (II-7) of the first wife died at 75 of broncho-pneumonia. While he was in his early fifties he had a period of severe stomach disorders. At the time he was "cancer conscious" and was certain he was afflicted, although the local physician had assured him he did not have cancer. However, he traveled to Salt Lake City and underwent surgery by a Salt Lake City physician. The operation revealed a duodenal ulcer. No trace of malignancy was found. A few years later he was treated and cured of a skin cancer on his nose.

The second wife (I-3) of the progenitor gave birth to two sons. The older son (II-8) lived until he was 89. He was free of disorders in his digestive tract with the exception of a short attack of ulcers when he was in his early fifties. The other son (II-9) lived for 78 years and was free from intestinal disorders.

The third wife (I-4) gave birth to seven children. The oldest son (II-10) lived until he was 80 and died from factors incident to old age. II-11 died at 38 years of influenza, and II-12 is still alive and in good health at 76 years. II-13 died of carcinomatosis in 1944 at the age of 69. She was always in good health until a few years before her death. She was treated for pernicious anemia and a year before her death an X-ray examination revealed a palpable epigastric tumor. She died from carcinoma of the stomach with generalized metastasis. II-14 died from influenza pneumonia at the age of 37.

The fourth wife (I-5) had eight children. The oldest child (II-17) is now 80 years old and in very good health. II-18 died at 37 of a cerebral hemorrhage. II-20 died at 65 years of age of surgical shock following an operation for cancer of the pyloris. Many years before his death he was troubled with ulcers which were apparently cured by proper diet. The symptoms returned and he was advised by the local physician to have an operation. The operation revealed extensive metastases which were inoperable. He died a few hours after the operation. II-21 died at 60 from carcinoma of the rectum with metastases to lungs and liver. His cause of death was verified by checking his death certificate and by interviewing the physician who attended him. II-23 died at 50 from a heart condition and II-24 is still alive and in excellent health. She is now 61 years old.

III-6 died at 46 following an operation for sarcoma of the kidney. He had never been seriously ill, at least he never complained very much. He spent the day before the operation herding cattle and doing other strenuous activities. The operation in a nearby hospital revealed he had hypernephroma of the right kidney and the malignancy of the tissue was confirmed by a pathological laboratory. He died the same day as the operation.

III-17 is now 61 years old. When she was 60 she noticed blood in her stools. Her family physician referred her to a specialist who found a small carcinoma 14 inches up her colon. It was removed surgically and its malignacy was confirmed.

II-21 died in Montana at the age of 53. Seven years before her death lumps were felt in her breast. Her physician took a biopsy and three weeks later removed the breast. A few years later the trouble reoccurred with metastasis to the lungs. Her death certificate states that she died from carcinoma of the lung.

Discussion

A kindred is presented which has a high concentration of cancer of the gastro-intestinal tract. Six members of the second generation have died from carcinoma of the stomach or lower region of the intestine. The father died from carcinoma of the inguinal region. In the third generation one member has recently had a portion of her colon removed in a cancerous condition, one member died from sarcoma of the kidney, and one female member died from carcinoma of the breast. The average age of death for those afflicted with all types of malignancies was approximately 61 years of age with a range from 46 to 75 years. The average age of death for those with carcinomas of the gastro-intestinal tract was approximately 62

with a range from 53 to 69 years. The period arbitrarily selected as the beginning of the cancer age for this kindred is 45 years of age.

Twenty-four children were born to I-1 and his four wives. Three of the families have had one or more members die from cancer. Six of the twenty-four children born to I-1 died in infancy, and three died under 45 years of age from causes other than cancer. Of the fifteen remaining, six died from carcinomas of the gastro-intestinal tract, one is questionable (II-5) and eight were unaffected. Benign neoplasms and skin cancers have not been included with these results.

The etiological role of heredity in this kindred is strongly suggested, but the exact nature of this genetical tendency cannot be shown at the present time. A large proportion of the members in the third generation have not yet reached the age at which cancer occurred in the other members of the kindred. Of the fifteen members of the second generation who were eligible for cancer, six were affected. The cancer incidence for this generation is 40 percent. At the present time 61 members of the third generation are over 45 years of age, but only three have been afflicted with a malignant growth. Three other members of the same generation are suspected cancer cases and are under observation by their physicians.

The available information does not demonstrate whether the high incidence of cancer in the second generation is due to a genetical tendency or merely a random assortment of cases. The present plan is to project the study into the future. If an inheritable organ-specific predisposition to cancer exists in this kindred, then the tendency will be expected to be present in future generations. The members of the third generation and other generations will be observed closely in following years for evidences of cancer.

Summary

A kindred is presented with a high incidence of carcinoma of the gastro-intestinal tract. The original progenitor was a polygamist with four wives. Gastro-intestinal cancer is found in three of the four families. The progenitor died from a melanotic growth on his leg which metastasised to the inguinal region. In the second generation four individuals were afflicted with carcinoma of the stomach and two with carcinoma of the rectum. So far in the third generation there has been one case of carcinoma of the colon, one breast carcinoma, and one kidney sarcoma. An inheritable organ-specific predisposition to cancer is suggested.

Literature Cited

1. Bell, J. *Treas. Hum. Inher.* 2:112-123. 1922.
2. Boehme, E. J. *Ann. of Surgery.* 131: 519-533. 1950.
3. Cockayne, E. A. Inherited Abnormalities of the Skin and its Appendages. pp. 274-288 Oxford Univ. Press, London. 1933.
4. Dukes, C. *Cancer Rev.* 5:241-256. 1930.
5. Gardner, E. J. and F. E. Stephens. *Jour. Human Genet.* 2:41-48. 1950.
6. Hoekstra, G. *Virchow's Arch.* 237-79-96. 1922.
7. Jacobsen, O. *Opera ex Domo Biol. Hered. Human. Univ. Hafn.* 11:1-306. 1947.
8. Martynova, R. P. *Am. J. Cancer.* 29: 530-540. 1937.
9. Penrose, L. S., H. J. MacKenzie, and M. N. Karn. *Ann. Eugen.* 14:234-266.
10. Smithers, D. W. *Brit. J. Cancer.* 2: 163-167. 1948.
11. Weller, C. V. *Cancer Res.* 1:517-535. 1941.

EVIDENCE OF A MULTIPLE SEX-CHROMOSOME MECHANISM

In a Gryllid

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THE sex-determining mechanism appears to be *XX-XO* in the majority of the species of gryllids studied so far. Although deviations from the usual *XO* mechanism to *XY* in the males are not altogether uncommon,[6, 10, 11, 12, 13] a multiple sex-chromosome mechanism is recorded only in a single species of Orthoptera Saltatoria *viz., Paratylotropidia brunneri* (Acrididae) by King and Beams[3]. We shall report in this paper an $X_1 X_2 : Y$ type of sex determination in an undetermined species of gryllid, belonging to the genus *Euscyrtus*. This is believed to be the first report of what seems to be a multiple sex-chromosome mechanism in Gryllidae. The most interesting aspect of the behaviour of the sex-chromosomes in this case is that they appear to be independent of meiotic pairing and orient themselves near the two poles of the spindle in the metaphase stage of the first division of meiosis—a phenomenon which has never been recorded in any Orthoptera studied so far.

Material and Methods

Testes of adult males and ovaries of immature females of *Euscyrtus* sp. were fixed either in Belling's modification of Nawaschin's mixture or in medium Flemming. Feulgen's nuclear staining method was used extensively. Some of the sections and smears were stained also in Heidenhain's haematoxylin and in crystal-violet following Newton's method.

The Chromosome Complement

The spermatogonial cells of the testis and the follicular epithelial cells of the ovary show at the metaphase stage 19 and 20 chromosomes respectively. (Figure 10*A*, *B* and *C* from the testis, *D* and *E* from the ovary).

The number of chromosome arms in this species could not be determined. It seems to vary not only in the chromosome complement of different individuals but also in the nuclei from the same individual. Figure 10*A* and *B* illustrate the variation in the spermatogonial cells of a single male specimen. Figure 10*A* shows four chromosomes bent in the middle two of which are among the longest and the other two belonging to the medium-sized chromosomes of the complement. In Figure 10*B* on the other hand, five V-shaped chromosomes are shown, one of which is lying in the center of the plate with its arms widely divergent. Figure 10*C* is drawn from a metaphase plate of a different male individual which shows three long and two medium-sized V-shaped chromosomes, and two other chromosomes which can be described as L-shaped. The number of V's also varies a great deal in the female. Figure 10*D* shows three large, two medium-sized and a small V chromosome; whereas Figure 10*E* shows five large V's. It should be noted, however, that actual constrictions are not visible in the regions where the chromosomes are bent in any of the preparations. Sometimes the chromosomes are bent in the form of a U. Thus the sharp and characteristic bends of the arms usually found at the constricted region of the chromosomes are sometimes absent in the various V-shaped chromosomes observed in this species.

Meiosis

The present material is not at all suitable for the study of the prophase stages of the spermatocyte division. Stages up to diakinesis are more or less diffuse. In earlier stages a positively heteropycnotic body (Figure 10*F* and Figure 9*A*) is always found which loses its identity by the time diakinesis is reached. Apart from this body, a big spherical nucleolus about one tenth the volume of the nucleus (Figure 10*F*) is also present. The heteropycnotic body lies quite independently of the nucleolus.

*The authors are indebted to Professor H. J. Muller for kindly going through the manuscript and suggesting valuable improvements. Our thanks are also due to Dr. B. P. Uvarov of the British Museum (Natural History) for the generic identfication of the material.

MICROPHOTOGRAPHS OF THE CHROMOSOMES OF *EUSCYRTUS*
Figure 9

A—Resting spermatocyte nucleus showing a single heteropycnotic body. *B*—Determinate disjunction of the sex chromosomes during metaphase I of meiosis. *C* and *D*—Two types of second meiotic metaphase cells with ten and nine chromosomes respectively.

The structure of the chromosomes can be clearly made out from the prometaphase stage of meiosis. Figure 10*G* shows a nucleus at that stage where eleven deeply stained bodies are clearly visible. The bivalent nature of eight of them is indisputable, whereas the other three appear to be univalents.

In the first spermatocyte metaphase stage the bivalents arrange themselves on the equatorial plate in the usual manner and the univalents, which are more or less spherical in shape, arrange themselves near the opposite poles of the spindle, two near one pole and one near the other (Figure 10*H* and Figure 9*B*).

It is surprising to find that when the chromosomes are viewed from the sides at this stage, they appear to be acrocentrics: the existence of a second arm cannot be detected in any one of them.

One of the three univalents is definitely bigger than the other two, the latter being almost of equal size. Their arrangement on the spindle is such that the largest one is always accompanied by one of the two small ones at one side of the equatorial plate, the other small chromosome remaining at the opposite side. This distribution is invariable and was determined from an examination of a large number of metaphase nuclei. When the chromosomes are viewed from the top at the above stage, the eight bivalents are seen in one plane and the remaining three univalents lie in different planes.

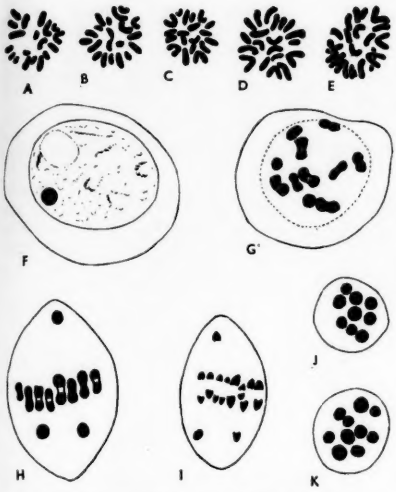
The first anaphase division (Figure 10*I*) is quite regular, the bivalents disjoin in the usual manner and move towards the opposite poles. The three univalents reach the nearer poles earlier than the rest of the chromosomes.

At the metaphase stage of the second meiotic division, two types of plates, one having nine and the other having ten chromosomes are observed (Figure 10*J* and *K*; Figure 9*D* and *C*). The chromosomes when viewed from the sides appear to be acrocentric. It may be recalled, in this connection, that no trace of a second arm was observed in the first meiotic division.

That the three univalents are the sex chromosomes can safely be suggested from their behavior during meiosis. Their characteristic distribution to the poles gives rise to two kinds of sperms and presumably half of them are male producing and the other half female producing. It has been stated earlier that during the first meiotic metaphase and anaphase stages they segregate in such a manner that the largest one is always accompanied by one of the smaller. Because there is no appreciable difference in size between the two smaller elements, it is not possible to distinguish one from the other. It might therefore be assumed that they are a pair of autosomes, functionally equivalent to one another, and that either one of them may accompany the large univalent chromosome. This however seems unlikely, especially in view of the similarity of their behavior to that of the large univalent. But if they are in fact a part of the sex-determining mechanism, they must be different from one another. In the latter case, in order that the sex-determining mechanism function properly, one of the small chromosomes, (that which remains alone on one side of the spindle) should be the *Y* chromosome and the other small one, together with the largest chromosome, at the other side, should be the *X*-complex. We should like to call the bigger of the two, X_1 and its partner X_2.

Discussion

Two difficulties confront us in determining the position of the centromeres in the chromosomes of *Euscyrtus* sp.



CHROMOSOMES OF *EUSCYRTUS*
Figure 10

Camera lucida drawings of the mitotic and meiotic chromosomes. *A* and *B* shows the variations in the number of V-shaped chromosomes in different spermatogonial cells of the same male individual. *C*—spermatogonial metaphase stage in another male specimen. *D* and *E* show the variation in the oogonial metaphase stage. *F* shows a spermatocyte resting stage with a single heteropycnotic body and a large nucleolus. *G* is a prometaphase of meiosis I showing eight bivalents and three univalents. *H* and *I* are metaphase and anaphase stages of the first meiotic division showing the segregation of the sex chromosomes. *J* and *K* represent the two kinds of second meiotic division metaphase stages showing nine and ten chromosomes.

First, the primary constrictions are not visible in any of the chromosomes and the bends between the two arms are sometimes not sharp. Second, the meiotic anaphase chromosomes, both at the first and the second divisions, do not show any trace of the presence of a second arm and hence all of them appear acrocentric. McClung and Asana[7] reported a number of J-shaped chromosomes in the spermatogonial metaphase plate of *Schizodactylus* but conclude from their variable appearance that they are really acrocentrics. Their conclusion is supported by the fact that the structure of the meiotic chromosomes shows clear evidence of their acrocentric nature. The evidence of the acrocentric nature of the meiotic chromosomes, on the other hand, cannot be conclusive in the case of *Euscyrtus* sp. This is obvious because throughout its meiotic stages the chromosomes are over-condensed and stumpy and consequently it would perhaps be impossible to detect the presence of the two arms of metacentric chromosomes in their over-condensed stage even though they were present. It is clear from the above that the position of the centromeres should be detected in this material from the structure of the mitotic chromosomes alone. The variable appearance of the latter stands in the way of determining accurately the number of metacentric chromosomes. Further work is necessary to clarify the issue.

Apart from the only case of multiple sex-determining mechanism in the Orthoptera Saltatoria[3], several such cases have been reported in Mantoidea[1,2,9]. In both the above groups of Orthoptera the prevalent type of sex determination is *XX-XO* and although in all probability the multiple mechanism in them has evolved directly from the *XX-XO* condition[14], a number of difficulties stand in the way of determining the evolution of multiple mechanism in *Euscyrtus* sp. First, the behavior of the chromosomes during meiosis cannot be observed accurately until near the prometaphase stage of the first division. Second, the sex chromosomes are uniformly stained throughout their length like the autosomes. Third, owing to the highly condensed condition of the chromosomes, it is impossible to determine the position of the centromeres in them, and last, the complete absence of prophase association and chiasma formation in the sex chromosomes makes it impossible to determine any homology between them. In consequence of the last, the mechanism of segregation of multiple sex-chromosomes in the first meiotic division in the male is different from those of the other Orthoptera. It is, on the other hand, like that of the

neuropteran sex chromosomes[4,8] where the X and Y chromosomes regularly segregate to opposite poles of the spindle without any previous association and chiasma formation. A more or less similar mode of segregation of the multiple sex-chromosomes is also found in various families of Heteroptera and also in various species of *Cimex*[14]. This phenomenon has been termed by Lorbeer[5] "distant conjugation" and by White[14] "determinate disjunction."

A case of determinate disjunction has been reported in another gryllid, *Gryllotalpa borealis*[11, 12] where presumably an unequal bivalent joined together by a single chiasma determines the segregation of a third univalent sex chromosome, the latter always going to the same pole with the larger component of the bivalent. The behavior of the sex chromosomes in the present case is quite different from that previously recorded because all the three chromosomes remain completely separate during meiosis and the Y chromosome apparently determines the disjunction of the X_1 and X_2 chromosomes from it or *vice-versa*.

Summary

1. The diploid chromosome number in *Euscyrtus* sp. is nineteen in the male and twenty in the female.
2. A varying number of V-shaped and L-shaped chromosomes are observed in mitotic metaphase both in the male and female and hence it is not possible to determine accurately the number of chromosome arms in the species.
3. It is inferred that this species has an X_1 X_2 Y mechanism of sex determination. At metaphase of the first division the eight bivalents arrange themselves on the equatorial plate and the other three chromosomes, although not forming any chiasma between themselves, disjoin regularly, those which we designate X_1 and X_2 going to one pole and Y to the other. X_2 and Y cannot be distinguished from one another, but their peculiar behavior, and its similarity to that of X_1 make it probable that they are not autosomes but part of a multiple sex chromosome system.
4. The above mode of segregation of the sex chromosomes is fundamentally different from that found in other Orthoptera and is recorded here for the first time.

Literature Cited

1. Asana, J. J. *Curr. Sci.* 2:244-245. 1934.
2. King, R. L. *J. Morph.* 52:523-533. 1931.
3. ———, and H. W. Beams. *J. Morph.* 63:289-300. 1938.
4. Klingstedt, H. *Faun. et Flor. Fenn.* 10:1-11. 1933.
5. Lorbeer, G. *Zahrb. wiss. Bot.* 80:567-817. 1934.
6. Makino, S. *J. Fac. Sci. Hokkaido.* 6:1-35. 1932.
7. McClung, C. E. and J. J. Asana. *J. Morph.* 55:185-191. 1933.
8. Naville, A. and J. de Beaumont. *Arch. Anat. Micr.* 29:199-243. 1933.
9. Oguma, K. J. *Coll. Agric.* Univ. Hokkaido. 10:1-27, 1921.
10. Ohmachi, F. *Proc. Imp. Acad. Japan.* 3:451-456. 1927.
11. Payne, F. *Archiv. f. Zellf. Bd.* 9:141-148. 1912.
12. ———. *J. Morph.* 28:287-327. 1916.
13. Voïnov, D. *Compt. Rend. Soc. Biol. Paris.* 76. 1914.
14. White, M. J. D. *Jour. Genet.* 40:303-336. 1940.